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RELATION OF THE NUMBER OF STREPTOCOCCUS LACTICUS TO THE AMOUNT OF ACID FORMED IN MILK AND CREAM *

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This study was carried on to determine whether or not a relation exists between the number of the streptococcus lacticus and the amount of acid formed in milk and cream. Observations on this question have been occasionally recorded, but systematic investigations have been scarce. Müller[1] found coagulation always took place at 37 C., while sometimes no coagulation occurred at 20 C., even tho a larger amount of acid was formed at 20 C. than at 37 C. Schröder[2] stated that acid fermentation may take place with greatly varying numbers of bacteria. Luxwolda[3] found, after many experiments, that the streptococcus lacticus grows best at 20 C. and that the coagulum formed at this temperature is solid and of pleasant taste. At higher and lower temperatures, competition of other bacteria is more in evidence and so the coagulum is not of desirable quality. Marshall's work[4] has shown that acid fermentation and coagulation are influenced in a measure by the presence of other bacteria, the activity of which may favor or retard multiplication of the streptococcus lacticus.

It seemed to me desirable to determine the amount of acid formed by the streptococcus lacticus in sterilized milk from day to day and at the same time record the number of bacteria present. It seemed also desirable to compare the results of acid fermentation and coagulation in raw milk in its natural state and of raw milk inoculated with the streptococcus lacticus.

A strain of the streptococcus lacticus with the following characteristics was used: individual cocci were round, gram-positive, and occurred chiefly in pairs, but chains of six to eight members were not uncommon. After numerous transfers, chain formation developed to a degree that chains of sixty to eighty

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[1] Centralbl. f. Bakteriol., Abt. 2, 1907, 17, p. 345.

[2] Original Dissertation, Dresden, 1908 (quoted after Luxwolda).

[3] Centralbl. f. Bakteriol., Abt. 2, 1911, 31, p. 129.

[4] Ibid., 1909, 24, p. 22.

members were observed. On dextrose agar a thin veil-like growth developed; on North medium the growth was similar, but more luxuriant. Litmus milk was coagulated within forty-eight hours at 37 C. and litmus decolorized, leaving a pink ring at the surface. After thirty-six transfers in litmus milk coagulation was complete in eighteen hours. Broth was rendered turbid with the accumulation of a diffusible sediment. Gelatin was not liquefied. When inoculated into 250 c.c. sterilized milk, a smooth coagulum was formed in twenty-four hours at 37 C. and a small amount of whey was separated. Nine Erlenmeyer flasks were used for each series. Three series were carried through, two series with milk, from which most of the cream had been removed, and one series with cream. In the first two series each flask was filled with 250 c.c. certified milk and in the last series with 250 c.c. certified cream. Three flasks of each series were sterilized in the autocleave for fifteen minutes under fifteen pounds' pressure. The organism was cultivated for twenty-four hours at 37 C. in tubes containing sterilized milk, and the whole contents of a tube was used for inoculation. The three flasks containing sterilized milk (or cream) and three of the flasks containing raw milk (or cream) were inoculated. Three flasks with raw milk and three with raw cream were allowed to sour spontaneously. The nine flasks of each series were incubated at three different temperatures, 37, 20, and 7 C.

The acidity of the milk and cream was determined before inoculation by titrating 5 c.c. diluted with 45 c.c. distilled water with 0.05 N.NaOH solution, with phenolphthalein as indicator. Plates were prepared both before and after inoculation to determine the number of bacteria present in the raw milk and the number that had resulted from inoculation. Every day thereafter for ten successive days, the amount of acid formed and the number of bacteria present were determined in the same manner. In the cream series, the work was continued for eight days. The culture medium used for plating was dextrose litmus agar of 1 percent acidity. Notes were made on taste, odor, and coagulation.

In Table 1, which gives the results of the experiments, the acidity is expressed in percent of N.NaOH. The numbers given for bacteria should be multiplied by 1,000. A clear view of the results is shown in the plotted curves. In the chart, the acidity is represented by the figures on the side in hundredths percent normal acid. The same figures give the numbers of bacteria in millions. The large dots indicate the approximate time when coagulation was complete. Figures at the bottom indicate the days of incubation.

In sterilized milk with no competition of other bacteria, there was a steady rise in acidity. After ten days the largest amount of acid was formed in milk kept at 20 C., while naturally the smallest amount was formed at 7 C. At 37 C. the milk was coagulated after about thirty-six hours with 540,000,000 bacteria. At room temperature coagulation took place after four days with 350,000,000 bacteria, and at 7 C. there was no coagulation visible after ten days. At 37 C. the number of bacteria decreased after the sixth day, but the acidity continued to increase. There was no decrease of bacteria in sterilized milk at 20 C. and at 7 C.

In milk not inoculated, the acidity curve ascended constantly for eight days, but the greatest amount of acid was formed at 37 C. Coagulation was complete after two days at 37 C. with 230,000,000 bacteria, but the number of bacteria decreased after this and then increased again after the sixth day. At 20 C. coagulation was complete after four days with 64,000,000 bacteria. The number rose to a maximum at seven days after which there was a decline. At 7 C. coagulation was complete after eight days with 136,000,000 bacteria. There was a decrease of numbers on the sixth day. In raw milk

TABLE 1

RELATION OF THE NUMBER OF LACTIC STREPTOCOCCI TO AMOUNT OF ACID PRODUCED UNDER DIFFERENT TEMPERATURE CONDITIONS*

Days	Temperature	Sterilized Milk Inoculated		Uninoculated Raw Milk		Raw Milk Inoculated	
		Acid in N NaOH	Bacteria† on Dextrose Litmus-Agar	Acid in N NaOH	Bacteria† on Dextrose Litmus-Agar	Acid in N NaOH	Bacteria† on Dextrose Litmus-Agar
0	37 C.	1.57	0	1.31	0	1.31	0
1		2.92	500	6.67	232	7.33	850
2		4.05	560	8.45	230	8.18	550
3		4.72	585	10.42	130	9.20	190
4		4.80	430	10.45	5	9.50	70
5		5.10	640	15.10	36	13.90	19
6		5.40	705	17.65	29	15.80	17
7		5.50	490	18.60	235	16.07	4
8		5.80	455	22.30	166	16.02	4
9		6.00	375	24.80	243	15.65	1.5
10		6.17	310	26.60	260	‡	‡
0	20 C.	1.57	0	1.31	0	1.31	0
1		1.57	63	1.23	0	1.63	5
2		1.65	255	1.30	14	3.65	46
3		1.85	390	3.65	32	6.50	77
4		2.00	350	7.60	64	7.90	98
5		2.35	930	7.80	106	8.02	120
6		3.02	1,300	8.05	221	8.50	75
7		4.25	1,335	8.80	267	8.70	57
8		5.70	3,150	9.10	105	9.00	39
9		7.10	1,980	9.35	80	9.17	21
10		7.22	2,600	9.65	54	9.70	17
0	7 C.	1.57	0	1.31	0	1.31	0
1		1.57	0	1.25	0	1.23	0
2		1.57	23	1.17	1	1.16	3
3		1.57	39	1.22	1	1.26	14
4		1.57	86	1.50	18	1.75	43
5		1.65	115	2.62	18	3.15	75
6		1.77	157	4.40	112	5.22	26
7		2.15	170	6.22	174	6.50	29
8		2.50	220	7.35	136	7.45	31
9		2.90	355	8.10	76	8.05	32
10		3.10	430	8.84	55	8.17	50

* Averages of the two series. ‡ Contaminated (mold).

† The numbers given for bacteria should be multiplied by 1,000.

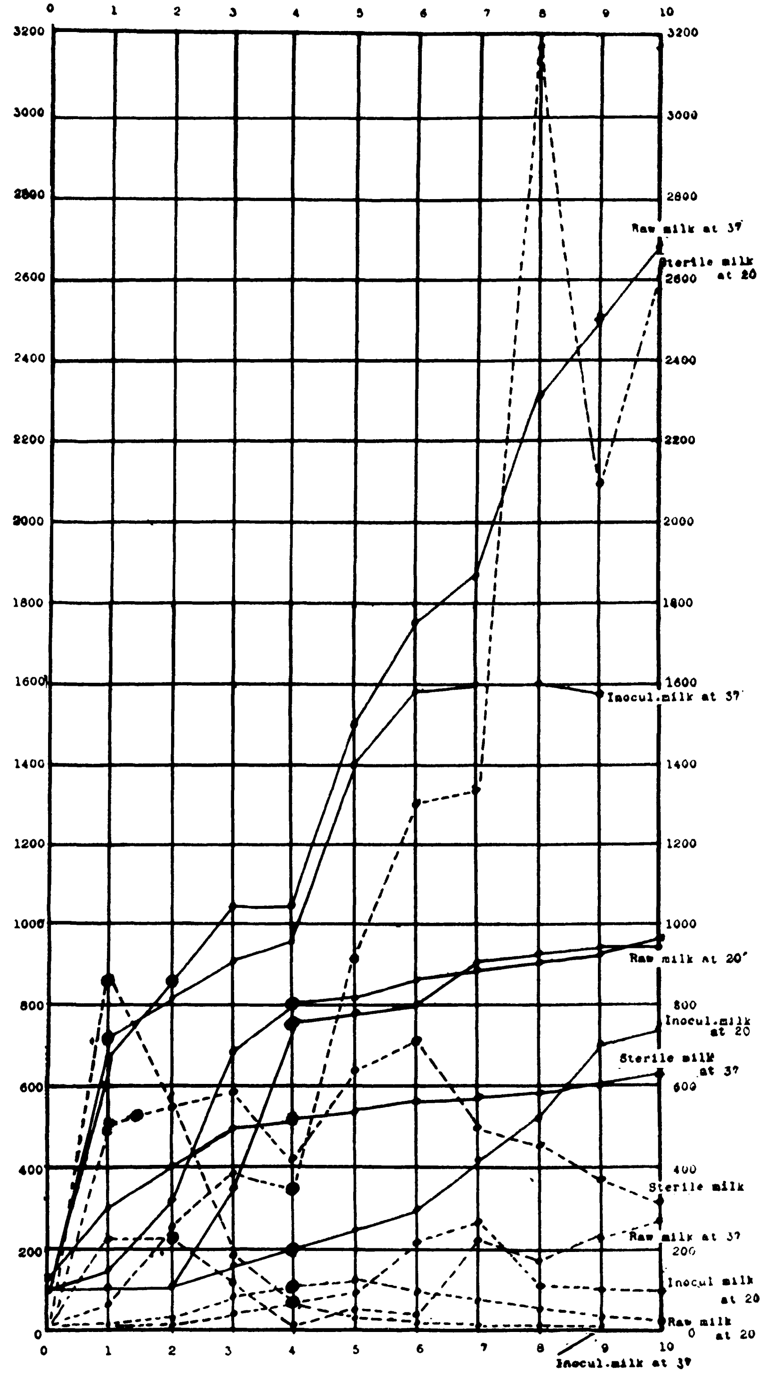

Chart 1.—Amount of acid formed in milk and number of bacteria present. Acid is repre-

inoculated with the streptococcus lacticus, there was a sharp rise of bacterial numbers reaching 850,000,000, after which there was a decided decline. The acidity also rose sharply and continued to rise to the eighth day, after which there was a slight decline. At 20 C. coagulation was reached on the fourth day with 98,000,000 bacteria. There was a slight rise for one day and then a decline. At 7 C. coagulation was complete on the seventh day with 29,000,000 bacteria.

At 37 C. inoculated raw milk was coagulated after twenty-four hours, sterilized milk after thirty-six hours, and raw milk not inoculated after two days. In sterilized milk, coagulation was complete with a smaller number of bacteria and with a smaller amount of acid than in inoculated raw milk, but more time was required for completion of the process. In raw milk not inoculated, the amount of acid present at the time of coagulation was higher than in either of the other milks, but the number of bacteria was relatively low. In both raw milks, enormous amounts of acid were formed at the end of the period of observation. In one case the amount was 26.6 percent (2.39 percent lactic acid), in the other 16 percent (1.44 percent lactic acid).

The amount of acid formed at 20 C. remained below 10 percent (0.9 percent lactic acid) to the end. The number of bacteria in sterilized milk inoculated with the streptococcus lacticus increased constantly with a small interruption from the eighth to the ninth day. In both raw milks a maximum of bacteria was reached, after which there was a decline. At 7 C. the acidity rose consistently with uninoculated milk in the lead, while sterilized milk acidified slowly. The number of bacteria in sterilized milk also rose consistently, while the number in both raw milks reached a maximum which was followed by a decline.

Several points of interest suggest themselves from the results. The enormous amount of acid formed at 37 C. in the raw milk is probably not due to the activity of the streptococcus lacticus alone. Market milk usually contains bacilli of the group of lacto-bacilli, which develop at relatively high temperatures and produce large amounts of acid. These bacteria grow abundantly after the streptococcus lacticus has stopped growing and they form very small colonies. It is probable that in counting colonies large numbers of these have been overlooked. They are difficult to see with small magnification, especially in the presence of other colonies. Examination of plates with a microscope during the later days of incubation showed that many were present. The assumption that the large quantity of acid formed in raw milk was not produced by the streptococcus lacticus alone is borne out by the fact that in sterilized milk inoculated with this organism the amount of acid remained relatively low.

A second point of interest is the fact that coagulation is not dependent solely on definite numbers of bacteria or a definite amount of acid. It is probable that acid-forming and coagulative enzymes, excreted by bacteria or liberated after disintegration of the cells, play an important rôle in bringing about coagulation. In sterilized milk inoculated with the streptococcus lacticus, the amount of acid increased after the number of bacteria had begun to decrease. This is true chiefly at 37 C., a temperature favorable to enzymic activity. The enzyme, which produces lactic acid from milksugar, is liberated by the large number of bacteria disintegrating and acts even if small numbers of bacteria are present. The large amount of acid produced at 37 C. may therefore be due to the action of enzymes or to the presence of lacto-bacilli, or in all probability to concerted action of both factors. A coagulative enzyme may also be active. The streptococcus lacticus is not known to produce such an enzyme, but, as has been shown by Marshall,[4] the presence of other bacteria commonly present in milk may aid in the production of acid or of a coagulum. Coagulation may thus be complete with relatively few bacteria present, or with a relatively small quantity of acid.

A third point is the fact that the number of bacteria reaches a maximum and then decreases. The higher the temperature the earlier the maximum is reached. The cause of this may be ascribed to the accumulation of metabolic products, notably acid. Conditions become unfavorable, multiplication stops, and the bacteria disintegrate. This phenomenon is more pronounced in raw milk, where many varieties of bacteria live in competition, than in sterilized, inoculated milk. But even here there is at 37 C. a distinct decrease after six days and at 20 C. after eight days. At 7 C. there is no decrease of numbers for ten days.

The foregoing discussion is based on the average of two series of experiments carried out with the same strain of the streptococcus lacticus, but with two different lots of milk. There is some difference in the number of bacteria and the amount of acid formed in the two series, but the general relation is similar. Generalization would be unjustified from these results as different strains of the streptococcus lacticus and different kinds of milk used as culture media might lead to different results. However, it may be assumed that the experiments give a fair picture of the phenomena occurring during the process of milk souring. This view is confirmed by the results of the work with cream. The figures are similar to those obtained with milk and warrant the same conclusions to be drawn.

CONCLUSIONS

The amount of acid formed in the souring process of milk or cream is not dependent solely on a definite number of bacteria of the streptococcus lacticus group. Temperature and the presence of other bacteria may influence the result.

In raw milk or cream, or in raw milk or cream inoculated with cultures of the streptococcus lacticus, the number of bacteria increases to a given point and then decreases. At 37 C. the maximum is reached after twenty-four hours and at lower temperature after several days.

Coagulation of milk or cream is not solely dependent on a definite amount of acid or a definite number of bacteria. This absence of definite relation between coagulation, on one hand, and acid and number of bacteria, on the other hand, may be due to the kinds of bacteria present; the kind of acid formed; and the activity of the enzymes produced by bacteria.

At 37 C. extraordinarily high amounts of acid may be produced after several days, due probably to the activity of enzymes produced by the streptococcus lacticus and to the presence of members of the group of lacto-bacilli.